Observations of time-based measures of flow-mediated dilation of forearm conduit arteries: implications for the accurate assessment of endothelial function

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1Division of Cardiology, Mount Sinai and University Health Network Hospitals, and 2Department of Pharmacology, University of Toronto, Canada; 3Department of Medicine II, University Hospital of Siena, Italy; and 4Department of Cardiology, University of Mainz, Germany

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Liuni A, Luca MC, Lisi M, Dragoni S, di Stolfo G, Mariani JA, Uxa A, Gori T, Parker JD. Observations of time-based measures of flow-mediated dilation of forearm conduit arteries: implications for the accurate assessment of endothelial function. Am J Physiol Heart Circ Physiol 299: H939–H945, 2010. First published July 16, 2010; doi:10.1152/ajpheart.00271.2010.—Endothelium-dependent flow-mediated dilation (FMD) is measured as the increase in diameter of a conduit artery in response to reactive hyperemia, assessed either at a fixed time point [usually 60-s post-cuff deflation (FMD60)] or as the maximal dilation during a 5-min continuous, ECG-gated, measure-ment (FMDmax-cont). Preliminary evidence suggests that the time between reactive hyperemia and peak dilation (time to FMDmax) may provide an additional index of endothelial health. We measured FMDmax-cont, FMD60, and time to FMDmax in 30 young healthy volunteers, 22 healthy middle-aged adults, 16 smokers, 23 patients with hypertension, 40 patients with coronary artery disease, and 22 patients with heart failure. As previously reported, FMDmax-cont was similar in healthy cohorts and was significantly blunted in smokers and all patient groups, whereas FMD60 was significantly blunted only in heart failure patients. There was a wide within-group variability between measures of time to FMDmax with no significant difference between normal and patient groups. Intra-arterial infusion of the nitric oxide synthase inhibitor Nω-monomethyl-arginine in eight healthy subjects resulted in a blunting of FMDmax-cont (P < 0.001) and FMD60 (P = 0.02) but not time to FMDmax. Both FMDmax-cont and FMD60 demonstrated good repeatability in 30 young healthy volunteers studied on two separate occasions (P < 0.01 for both), whereas time to FMDmax varied widely between visits (P ≠ significant). In conclusion, although time to FMDmax does not appear to be a useful adjunctive measure of endothelial health, the use of continuous diameter measurements provides important data in the study of endothelial function in healthy subjects and patients with cardiovascular disease.

endothelium; vascular physiology; flow-mediated dilation; arterial diameter; time to peak dilation

FLOW-MEDIATED DILATION (FMD) is a noninvasive technique used to evaluate the function of the vascular endothelium in peripheral conduit arteries. The concept of FMD is based on the observation that the induction of ischemia in a peripheral limb (usually the hand/forearm), followed by rapid deflation of the pneumatic cuff used to induce this ischemia, causes a sudden increase in blood flow (reactive hyperemia) in the conduit artery that provides blood to this territory. The ensuing sudden change in local shear stress causes an endothelium-dependent production of a number of vasoactive substances, including nitric oxide (NO) (5, 7). Since the ability of the vessel to respond to changes in shear stress is dependent on an intact and healthy endothelium, the magnitude of this flow-induced vasodilatory response has been proposed as a surrogate measure of overall endothelial function. Importantly, FMD of the radial artery has been demonstrated to be mediated primarily by NO, such that the response observed can also indicate the degree of NO bioavailability (23), although this concept has been recently challenged (26).

Traditionally, FMD is calculated as the dilation induced by reactive hyperemia, expressed in millimeters or percent change from resting diameter at either 60 s (FMD60) or within a relatively narrow time range following cuff deflation. Measured this way, FMD correlates with coronary endothelial function (1) and is blunted in a number of cardiovascular conditions, including coronary artery disease (CAD), hypertension (HTN), and heart failure (HF), as well as in preclinical subjects with risk factors for atherosclerosis (21, 23, 24). However, the use of a fixed time frame for FMD measurements has been recently questioned with a study demonstrating that the arterial diameters observed at 60 s underestimated the true maximal FMD response (3). Furthermore, in an attempt to better characterize the capacity of the vascular endothelium to react to changes in shear stress, other parameters of the FMD response have been proposed, including the measurement of the time to peak dilation [i.e., time difference between the induction of reactive hyperemia and the peak dilatory response (time to FMDmax) see Fig. 1]. Interestingly, Black et al. (3) recently showed that time to FMDmax is significantly shorter in young compared with healthy middle-aged volunteers.

The present study investigated 1) whether there are differences in repeatability and NO dependency between different measures of FMD, 2) whether time to FMDmax is modified during infusion of a NO synthase inhibitor and whether it is a repeatable measure, and 3) whether the method used to measure FMD influences the capacity to distinguish between healthy subjects and patients with CAD, HTN, or HF or smokers.

MATERIALS AND METHODS

The ethics committees of the Mount Sinai Hospital (Toronto), the University Medical Center Mainz, and the University of Siena ap-
proven measurements of FMD in healthy volunteers and patients with cardiovascular disease. Written, informed consent was obtained in all cases. All studies were performed between 11:00 AM and 2:00 PM. Participants were in a fasted state for at least 6 h before the study.

Measurement of arterial diameter and time to FMDmax. The methods for the assessment of FMD in our laboratory, as well as the repeatability of measurements, have been previously described in detail (11, 12, 17, 18, 20). Briefly, end-diastolic, ECG-gated, longitudinal, B-mode images of the artery 10–15 cm below the antecubital fossa were digitally acquired and stored for off-line analysis. Arterial diameter was recorded continuously for 1 min before cuff inflation (resting diameter), during the period of distal cuff inflation (4'30''), and for another 4'30'' after wrist cuff deflation. Semiautomated, custom-designed software that allowed for human correction was employed to calculate the arterial diameter from the trailing edge to the leading edge of the interface between the intima and blood. Three parameters were considered: 1) FMDmax-cont, i.e., the maximum percent increase in arterial diameter in the 4'30'' following cuff deflation compared with resting diameter; 2) FMD60, i.e., the percent increase in arterial diameter at 60 s after wrist cuff deflation compared with resting diameter; and 3) time to FMDmax, i.e., the time interval from wrist cuff deflation to maximal arterial diameter (see Figure 1 for all).

All data were analyzed in a randomized, blinded fashion after FMD data files were coded by laboratory staff not involved in data acquisition or analysis. Shear rate was calculated (in s−1) as blood velocity/radial artery diameter (27).

Repeatability of the methods. For repeatability studies (variability between occasions), 30 healthy young volunteers (18–34 yr of age) underwent measurement of FMDmax-cont, FMD60, and time to FMDmax on two separate occasions, separated by ≥24 h. In these subjects, the exact location of the probe was marked on the skin, allowing the same imaging site to be used during each of the measurement periods.

The role of NO in FMD and time to FMDmax. FMDmax-cont, FMD60, and time to FMDmax were measured during the intra-arterial infusion of normal saline, followed by a 30-min washout period, and subsequently during infusion of the NO synthase inhibitor Nω-nitro-L-arginine (L-NMMA; 8 μmol/min) in eight healthy male volunteers (18–25 yr of age) (16). Infusions were started 10 min before radial artery diameter recording. Because of the long half-life of L-NMMA, the order of the infusions was not randomized. However, preliminary studies from our laboratory (data not shown) and others showed that FMD measurements can be repeated at an interval of 30–60 min without significant variability (8, 13).
lation coefficient was in this case 0.6 (Fig. 2C, \( P < 0.01 \)), and the coefficient of variation was 29%. In contrast, we found a large degree of variability in time-to-FMD\(_{\text{max}}\) measurements. There was a nonsignificant correlation between repeated measurements of this variable (Fig. 2E, intraclass correlation coefficient = 0.1, \( P = 0.3 \)). The coefficient of variation for these data was 35%. The Bland-Altman plot for repeated FMD\(_{\text{max-cont}}\) values is shown in Fig. 2B. The bias calculated in absolute differences was 1.4% with 95% limits of agreement between 3.8 and 3.9%. The mean FMD\(_{\text{max-cont}}\) value is 6.4 ± 2.6%. The mean FMD\(_{60}\) value is 3.6 ± 3.0%. The limits of agreement for FMD\(_{60}\) are between −4.6 and 5.0%. The limits of agreement are between −95 and 85 s for time to FMD\(_{\text{max}}\). The mean time to FMD\(_{\text{max}}\) value is 59.3 ± 35.0 s.

The role of NO in FMD\(_{\text{max-cont}}\), FMD\(_{60}\), and time-to-FMD\(_{\text{max}}\) measurements for consecutive FMD procedures in healthy volunteers. FMD\(_{\text{max-cont}}\) (A), FMD\(_{60}\) (C), and time to FMD\(_{\text{max}}\) (E) were evaluated for FMD experiments performed ≥24 h apart in the same subject. The curves illustrate the correlation between the FMD\(_{\text{max-cont}}\), FMD\(_{60}\), and time-to-FMD\(_{\text{max}}\) measurements [intraclass correlation coefficient (ICC) = 0.7, \( P < 0.01 \); ICC = 0.6, \( P < 0.01 \); and ICC = 0.1, \( P \) not significant, respectively]. B, D, and F: Bland-Altman curves of repeatability. The average values of the 2 corresponding FMD\(_{\text{max-cont}}\), FMD\(_{60}\), and time-to-FMD\(_{\text{max}}\) values are plotted against the difference between the same values. The limits of agreement for FMD\(_{\text{max-cont}}\) are between −3.9 and 3.8%. The mean FMD\(_{\text{max-cont}}\) value is 6.4 ± 2.6%. The mean FMD\(_{60}\) value is 3.6 ± 3.0%. The limits of agreement for FMD\(_{60}\) are between −4.6 and 5.0%. The limits of agreement are between −95 and 85 s for time to FMD\(_{\text{max}}\). The mean time to FMD\(_{\text{max}}\) value is 59.3 ± 35.0 s.

Infusion of L-NMMA caused a significant blunting of baseline radial artery blood flow without altering resting diameter, resulting in a significant percent increase in blood flow after deflation (Table 1). FMD\(_{60}\) and FMD\(_{\text{max-cont}}\) were both blunted during L-NMMA compared with placebo (respectively, from 4.6 ± 3.0 to 0.9 ± 2.6% and from 7.2 ± 2.1 to 2.7 ±
1.9%, corresponding to a mean blunting of 80.1% in FMD$_{60}$ and of 64.7% in FMD$_{\text{max}}$-cont). The impact of L-NMMA on the two variables was not statistically different. In contrast, L-NMMA had no effect on the time to FMD$_{\text{max}}$ (Table 1 and Fig. 3).

**FMD$_{\text{max}}$-cont, FMD$_{60}$, and time to FMD$_{\text{max}}$ measurements in healthy volunteers and patients with cardiovascular disease.** In the CAD group, 35 patients also had HTN, 38 had hypercholesterolemia, and 15 had diabetes mellitus. None of them had systolic or diastolic HF. All CAD patients were taking aspirin. As previously published by our group and others, baseline blood flow, blood flow after cuff inflation, and reactive hyperemia were similar in all groups. FMD$_{\text{max}}$-cont was similar between healthy young and healthy middle-aged volunteers, and it was significantly blunted in all patient groups (healthy young, 7.3 ± 3.0%; healthy middle-aged, 6.1 ± 4.3%; smokers, 4.2 ± 4.7%; HTN, 3.2 ± 2.0%; CAD, 3.6 ± 2.5%; and HF, 2.7 ± 3.6%; P < 0.01 for all patient groups). FMD$_{60}$ values were similar in the normal volunteer groups (healthy young and middle-aged) but were blunted in all patient groups (healthy young, 4.8 ± 3.9%; healthy middle-aged, 3.5 ± 4.8%; smokers, 1.9 ± 5.0%; HTN, 1.3 ± 2.5%; CAD, 2.0 ± 2.5%; and HF, 0.5 ± 3.4%). The differences in FMD$_{60}$ between the healthy middle-aged cohort and the various patient groups, however, reached statistical significance only in the case of the HF group (P < 0.001 compared with healthy middle-aged volunteers). The time to FMD$_{\text{max}}$ was not found to be significantly different between any of the groups (healthy young, 61.4 ± 34.0 s; healthy middle-aged, 75.9 ± 33.0 s; smokers, 59.0 ± 29.6 s; HTN, 73.2 ± 34.7 s; CAD, 64.4 ± 45.0 s; and HF, 86.0 ± 42.8 s). In healthy volunteers, regression analysis showed a weak but statistically significant linear correlation between age and time to FMD$_{\text{max}}$ (Fig. 4). There was no relationship between age and the other parameters of FMD.

**Heterogeneity of time to FMD$_{\text{max}}$ measurements.** In young healthy subjects and healthy middle-aged adults, time to FMD$_{\text{max}}$ varied between 17 and 143 s and between 30 and 143 s, respectively. The time to FMD$_{\text{max}}$ in smokers ranged 22–134 s. In patients with HTN, CAD, and HF, the time-to-FMD$_{\text{max}}$ values ranged 26–150, 14–187, and 13–172 s, respectively.

**DISCUSSION**

FMD has been previously expressed as the percent increase in conduit artery diameter at 60 s following induction of local reactive hyperemia compared with resting conditions. The prognostic impact of such fixed time-point measurements of FMD has been clearly established (14, 15, 28, 29). More recent investigations have called into question the use of measurements at a fixed time point, which may underestimate the true maximal dilatory response (3). A number of other parameters can be derived from the analysis of the response of a conduit artery to reactive hyperemia. Among these, recent studies have focused on the time course of the FMD response and particularly on the measurement of the time from deflation of the pneumatic cuff to peak arterial diameter (time to FMD$_{\text{max}}$). However, it remains unclear whether this “time domain” pa-

### Table 1. Radial artery diameters and blood flow changes during saline and L-NMMA infusions

<table>
<thead>
<tr>
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<th>Normal Saline</th>
<th>L-NMMA</th>
<th>P</th>
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<tr>
<td>Radial artery diameter, mm</td>
<td>2.42 ± 0.32</td>
<td>2.42 ± 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum change in diameter postdeflation</td>
<td>0.17 ± 0.04</td>
<td>0.06 ± 0.04</td>
<td>&lt;0.001</td>
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<tr>
<td>Change in diameter at 60 s postdeflation</td>
<td>0.11 ± 0.07</td>
<td>0.02 ± 0.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Time to FMD$_{\text{max}}$, s</td>
<td>61 ± 22</td>
<td>91 ± 67</td>
<td>NS</td>
</tr>
<tr>
<td>Radial artery blood flow</td>
<td>Resting blood flow, ml/min</td>
<td>18 ± 11</td>
<td>9 ± 5</td>
</tr>
<tr>
<td>Increase in blood flow after cuff deflation, %</td>
<td>843 ± 307</td>
<td>1,631 ± 620</td>
<td>&lt;0.01</td>
</tr>
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Values are means ± SD. L-NMMA, N$^\text{\textsuperscript{\text{-}}}$-monomethyl-L-arginine; NS, not significant.

Fig. 3. FMD$_{\text{max}}$-cont, FMD$_{60}$, and time-to-FMD$_{\text{max}}$ measurements during the infusion of NS and N$^\text{\textsuperscript{\text{-}}}$-monomethyl-L-arginine (L-NMMA). Values of FMD$_{\text{max}}$-cont were significantly lower following infusion of L-NMMA (P < 0.001). FMD$_{60}$ measurements were also significantly lower following L-NMMA infusion (P = 0.02). Measurements of time to FMD$_{\text{max}}$ were not significantly different after L-NMMA infusion. NS, normal saline (where shown on x-axes of graphs) or not significant (where shown as P value).
FMDmax-cont, flow-mediated dilation calculated using maximum arterial diameter after cuff deflation measured continuously; FMD60, flow-mediated dilation calculated using measurement of arterial diameter at 60 s after cuff deflation.

Table 2. Sample size estimates for FMDmax-cont and FMD60: total sample size required

<table>
<thead>
<tr>
<th>Effect Size, %Baseline</th>
<th>Crossover Design</th>
<th>Parallel Design</th>
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<tbody>
<tr>
<td></td>
<td>FMDmax-cont</td>
<td>FMD60</td>
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<tr>
<td>10</td>
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FMDmax-cont, flow-mediated dilation calculated using maximum arterial diameter after cuff deflation measured continuously; FMD60, flow-mediated dilation calculated using measurement of arterial diameter at 60 s after cuff deflation.

Fig. 4. Regression analysis for time to FMDmax and age in healthy volunteers. Neither FMDmax-cont values nor FMD60 were significantly related to age. In contrast, time to FMDmax had a significantly positive correlation with age (P < 0.05).
difference between time-to-FMD\textsubscript{max} values in cohorts of young and older healthy subjects, suggesting that this observation may have been due to the change in arterial compliance that accompanies advanced age (3). If confirmed, these data might support the concept that an increased vascular stiffness might delay, but not blunt, FMD. These hypotheses deserve further investigations.

**Within-group heterogeneity of time to FMD\textsubscript{max}**. Our data show that time to FMD\textsubscript{max} is highly heterogeneous in healthy subjects as well as smokers and patients with cardiovascular disease (HTN, CAD, and HF). These data, which expand on the previous findings of Black et al. (3) in healthy subjects and those of Palinkas et al. (25) in patients with CAD, indicate that the peak vasodilatory response occurs over a broad time period and suggest that the use of a limited time period for the measurement of arterial diameter (e.g., FMD\textsubscript{60}) systematically underestimates the response to the hyperemic stimulus, capturing the peak FMD response in very few individuals (Fig. 3). Thus our data suggest that continuous (ECG gated) analysis of arterial diameter after cuff deflation is able to detect the true peak FMD, improving the signal-to-noise ratio of the method.

**Between-group comparisons of FMD\textsubscript{max-cont}, FMD\textsubscript{60} and time to FMD\textsubscript{max}**. It has been well established that endothelium-mediated responses such as FMD are significantly lower in patients with cardiovascular disease as well as in subjects with risk factors for atherosclerosis such as smoking and hypercholesterolemia (5, 21, 23, 24). Our data indicate that whereas the peak vasomotor response to shear stress-mediated release of endothelial vasoactive mediators is impaired, the temporal kinetics of this response is highly variable and not different in the setting of health versus those who smoke or have overt cardiovascular disease. In an analysis of low-risk subjects, defined by a 10-yr Framingham risk score of <10%, Chironi et al. (6) found that time to FMD\textsubscript{max} was not associated with Framingham risk score or carotid artery intima-media thickness. Donald et al. (9) similarly demonstrated no difference in time to FMD\textsubscript{max} between healthy control subjects and patients with type 2 diabetes mellitus or hypercholesterolemia. Our findings complement these observations, since they are the first to demonstrate that time to FMD\textsubscript{max} is not altered in the setting of smoking, HTN, CAD, or HF and is not a robust measure of endothelial health and cardiovascular status. The large heterogeneity of time to FMD\textsubscript{max} also appears to play a role in the reduced sensitivity of FMD\textsubscript{60} in detecting alterations in smokers, HTN, CAD, and HF compared with the measurement of FMD\textsubscript{max-cont}. While FMD\textsubscript{60} and FMD\textsubscript{max-cont} both were lower in the disease groups, in the case of FMD\textsubscript{60} (age adjusted) statistical significance was reached only in HF patients, a difference that is compatible with the high variability in the time kinetics of the vasodilatory response. A number of limitations need to be acknowledged, such as the relatively small sample size and the use of a specific FMD setup (ECG gating, radial vs. brachial or coronary arteries, and distal vs. proximal cuff, etc.). Also, since our goal was to compare different measures of FMD, we did not assess nitroglycerin-induced dilation; thus it is possible that impaired smooth muscle responsiveness may have contributed to our observed differences between healthy subjects and the patients populations studied.

Our results demonstrate that time to FMD\textsubscript{max} is unable to differentiate between healthy controls and those with compromised cardiovascular health, and that, unlike other FMD measures, is not influenced by NO synthase blockade. This observation suggests that time to FMD\textsubscript{max} does not provide additional information that is complementary to traditional FMD in studying endothelial function in patients with cardiovascular risk factors or overt cardiovascular disease. Furthermore, the heterogeneity of time-to-FMD\textsubscript{max} measures across healthy volunteers and patients with cardiovascular disease increases the variability and decreases the sensitivity of FMD\textsubscript{60} in detecting differences in patient cohorts, as shown by our power calculations. Collectively, our data emphasize the importance of prolonged measurements of arterial diameter throughout the entire FMD procedure.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


